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(54) Title: A PROCESS FOR PRODUCING A COAGULATION ACTIVE COMPLEX OF FACTOR VIII FRAGMENTS <div style="display: flex; justify-content: space-between;"> <div data-bbox="215 1197 852 1999" style="width: 55%;"> (57) Abstract <p>A coagulation active complex of Factor VIII fragments is produced by causing a coagulation inactive FVIII heavy chain to react with a coagulation inactive FVIII light chain in the presence of a complex forming agent. Thus, FVIII-HC and FVIII-LC are converted to coagulation active FVIII complex in the presence of metal ions, such as Mn²⁺, Ca²⁺ or Co²⁺ or a component of the prothrombin complex or a substance having reactivity to compounds containing the group -SH and/or -S-S.</p> </div> <div data-bbox="852 1197 1507 1999" style="width: 40%; text-align: center;"> <table> <thead> <tr> <th>1</th> <th>2</th> <th>3</th> <th>kD</th> </tr> </thead> <tbody> <tr> <td></td> <td></td> <td></td> <td>205</td> </tr> <tr> <td></td> <td></td> <td></td> <td>116</td> </tr> <tr> <td></td> <td></td> <td></td> <td>95</td> </tr> <tr> <td></td> <td></td> <td></td> <td>66</td> </tr> <tr> <td></td> <td></td> <td></td> <td>45</td> </tr> <tr> <td></td> <td></td> <td></td> <td></td> </tr> </tbody> </table> </div> </div>			1	2	3	kD				205				116				95				66				45				
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A process for producing a coagulation active complex
of Factor VIII fragments

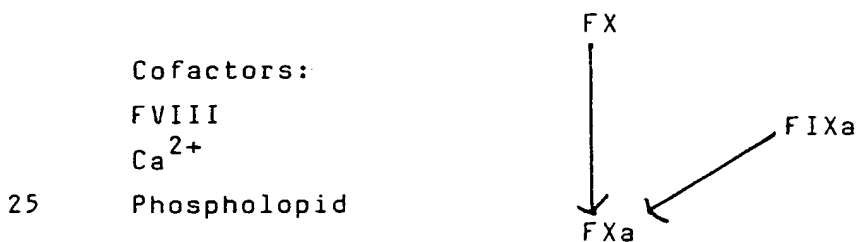
5 The present invention concerns a process for producing
a coagulation active complex of an N terminal fragment
of Factor VIII with a molecular weight of 92 to 210
kd and a C terminal fragment of Factor VIII with a
molecular weight of 80 to 70 kd.

10

Factor VIII is a protein occurring naturally in blood.

It participates as a cofactor in the conversion of FX
to activated FX (FXa). The presence of FVIII increases
15 the FXa generation rate about 200,000 times (Dieijen
et al, J. Biol. Chem. 156, p. 3433, 1981). Lack of FVIII
(hemophilia A) manifests itself as uncontrolled bleedings.

The role of FVIII in the coagulation cascade appears
20 from the following scheme:



FVIII may be activated by thrombin or FXa and be in-
activated by thrombin, FXa or protein C.

30 Hemophilia A patients are treated with FVIII prepara-
tions, either prophylactically or acutely in case of
bleedings.

FVIII can be recovered from human blood plasma in which
35 about 1 ppm of the protein is FVIII. This method can
only produce limited amounts of FVIII, and it is therefore

desirable to produce FVIII biosynthetically in cell culture. Three groups of researchers have been successful in doing this (Wood et al, Nature 312, p. 330, 1984 - Toole et al, Nature 312, p. 342, 1984 - Truet et al, 5 DNA 4, p. 333, 1985).

2 mg of foreign protein/ml can be produced in cell culture. For FVIII this would correspond to 20,000 units/ml. This level far exceeds what has been described for FVIII 10 in the literature. One of the reasons is that FVIII is a very large protein with a molecular weight of about 330 kd (Vehar et al, Nature 312, p. 337, 1984).

FVIII purified from blood plasma or from cell culture 15 comprises a fragment called Factor VIII light chain or FVIII-LC with a molecular weight of about 80 kd, and a fragment called FVIII heavy chain or FVIII-HC with a molecular weight of from 92 to 210 kd. The fragments are produced from the 330 kd protein by proteolytic 20 cleavage so that 80 kd FVIII-LC is the C terminal fragment, while FVIII-HC is the N-terminal fragment whose size depends upon the degree of cleavage.

Thus, it is known that coagulation active FVIII includes 25 an 80 kd fragment and a fragment with a molecular weight of 92 kd or more (Fulcher and Zimmerman, Symposium on FVIII, Scripps Clinic, 1982).

It would be an advantage to produce FVIII from smaller 30 fragments if these fragments in vitro could be combined to coagulation active FVIII. The fragments could more easily be produced biosynthetically in large amounts because of their smaller size with respect to intact FVIII. Further, the production of fragments of FVIII 35 would be advantageous with a view to subsequent purification from cell culture as the product changes charge

and molecular weight by the complex formation. Since it is thus possible to purify the fragments as well as the complex, a purer end product may be obtained.

5 Experiments have shown that simple combination of the fragments does not provide coagulation active products. Nor does the literature describe methods or conditions which would enable fragments of FVIII to be converted to a coagulation active complex.

10

The present invention is based on the finding that a coagulation active complex is produced when a coagulation inactive FVIII-heavy chain (FVIII-HC, N-terminal fragment) is caused to react with a coagulation inactive FVIII-light
15 chain (FVIII-LC, C-terminal fragment) in the presence of a complex promoting agent. This is quite surprising because the literature reports purification of FVIII-LC and FVIII-HC, cf. DK Patent Application 5387/85, without reporting any attempted activity creating combination,
20 notwithstanding that activity creation by combination might have great theoretical and practical importance.

Burke et al (Abstract 14 p. 111. Research in clinic and laboratory 16, 1986) have been able to produce coagu-
25 lation active FVIII in vivo with cells transfected with DNA for both FVIII-LC and FVIII-HC. However, it was not possible to obtain coagulation activity by mixing culture supernatants which contained FVIII-LC and FVIII-HC, respectively, even though various conditions were tried.

30

FVIII-LC can be purified from human plasma and has no coagulation activity (see WO 86/02838). FVIII-HC fragments can also be purified from blood plasma (Truett et al, DNA 4, p. 333, 1985). Also these fragments are without
35 coagulation activity.

According to the invention, one or more divalent metal ions are preferably used as complex promoting agents. Examples of suitable agents of this type are Mn^{2+} , Ca^{2+} and Co^{2+} . Other suitable complex promoting agents are
5 FIXa and FX and substances having reactivity to R-SH and/or R-S-S-R compounds. If desired, a mixture of these agents may be used.

DEFINITIONS

10

FVIII-LC or FVIII light chain is a fragment from the C terminal domain of full length FVIII. The molecular weight of the fragment is typically about 80 kd, but may be 70 kd or less. The fragment with the molecular
15 weight of 80 kd has immunological reactivity in the described assay for FVIII-LC antigen and not in assay for FVIII-HC antigen.

FVIII-HC or FVIII heavy chain is a fragment from the
20 N terminal domain of full length FVIII. The molecular weight of the fragment is typically 92 kd, but may be less and up to 210 kd. FVIII-HC purified from plasma consists of a mixture with a molecular weight of 92 to 210 kd. The fragment with a molecular weight greater
25 than or equal to 92 kd has immunological reactivity in the described assay for FVIII-HC antigen but not in assay for FVIII-LC antigen.

Coagulation active FVIII is a protein which is capable
30 of reducing the coagulation time of hemophilia A plasma in coagulation assay. Coagulation active FVIII is moreover capable of promoting the formation of FXa in Coatest assay (cf. the following) and thus of converting the chromogenic substrate. Coagulation activity is stated
35 as FVIII:C.

Prothrombin complex is coagulation factors containing γ -carboxyglutamic acid, i.e. FII, FVII, FIX, FX protein C or activated forms of these coagulation factors (Davie et al, Advances in enzymology 48 p. 277, 1979).

5

METHODS

Coatest assay for FVIII:C

10 In this assay, FVIII:C is measured in a system consisting of FIXa, FX, Ca^{2+} and phospholipid (PL) (Rosen et al, Thromb Haemostas 54 p. 818, 1985). FXa is formed in an amount depending upon the amount of FVIII:C. The assay is performed as indicated below:

15

Coatest assay for FVIII:C

1. A 50 μ l sample is mixed with 50 μ l of activation reagent (mixture of FIXa/FX and PL). Incubation time
20 10 min., 22°C.
2. 25 μ l of 25 mM CaCl_2 is added, and the mixture is incubated for 20 minutes at 22°C.
- 25 3. 50 μ l of chromogenic substrate (S2222) for FXa is added.
4. After incubation for 15 min. citric acid is added, and E_{405} of the sample is read.

30

It is not possible in the Coatest assay to follow an enzymatic activation of FVIII because FVIII in the assay is activated fully by incubation with FIXa/FX, PL and Ca^{2+} .

35

Immunological quantization of FVIII-LC

FVIII-LC antigen (Ag) is measured in specific immuno-
assay (Nordfang et al, Thromb Haemostas 53, p. 346,
5 1985). Human inhibitor antibody is coated to microplates,
sample is added, and bound FVIII-LC is detected with
peroxidase labelled F(ab')₂ fragment of human inhibi-
tor IgG. Normal human plasma is used as a standard.

10 Immunological quantization of FVIII-HC

FVIII-HC antigen (Ag) is measured in specific inhibition
assay. Dog inhibitor antibody is coated to microplates
with loose wells. Sample and ¹²⁵I-labelled FVIII-HC
15 are added. The amount of FVIII-HC in the sample determines
the amount of bound ¹²⁵I-FVIII-HC. The standard is FVIII
concentrate (FVIII Nordisk) set to contain 1 FVIII-HC
unit per FVIII:C unit.

20 The amount of FVIII-LC and FVIII-HC determined by immuno-
assay is stated relatively. That is the proportion between
unit FVIII:C unit FVIII-LCAg unit FVIII-HCAg for
various types of FVIII is not 1:1:1. However, it is
assumed that units of the various assays are comparable,
25 but there may be some difference between VIII:C unit,
FVIII-LCAg unit and FVIII-HCAg unit for a FVIII sample
in which all protein is coagulation active in Coatest.

Determination of molecular weight

30 Molecular weight is determined by reduced SDS-PAGE
(Laemmli, Nature 227 p. 680, 1970).

Production of FVIII

35 FVIII sample for control tests was produced from FVIII

concentrate (see WO 84/03628) by affinity chromatography on goat anti-von Willebrand Factor Sepharose (Truett et al, DNA 4 p. 333, 1985).

5 Preparation of FVIII-LC (sample A)

FVIII-LC may be purified from blood plasma by several methods (as described e.g. in WO 86/02838). Here, highly concentrated FVIII-LC is used, isolated by affinity
10 chromatography on monoclonal 47 IgG of Nordiocto, produced as described by O. Nordfang et al.: Thrombosis and Haemostasis, Vol. 54, p. 586-590, 1985. Nordiocto, dissolved in 200 ml of buffer A (0.02 M imidazole, 0.15 M NaCl, 10 mM EDTA, pH 7.4) to a concentration of VIII-LCAg
15 of 110 units/ml, was incubated overnight with 7 ml of 47 IgG sepharose (coupled with 9 mg of IgG/ml). The incubation mixture was poured on a column, and eluate was collected. The gel was washed with 40 ml of buffer A and 40 ml of buffer A with a total of 0.65 M NaCl.
20 FVIII-LC was eluted with 40 ml of 20 mM imidazole/0.65 M NaCl/10 mM EDTA/50% ethylene glycol/pH 7.4. A peak fraction of 4 ml was dialysed to 50 mM imidazole/0.15 M NaCl/10% glycerol/0.02% NaN₃/pH 7.4. The content of FVIII components in the dialysed sample appears from
25 table 1.

Preparation of FVIII-HC (sample B)

FVIII-HC is produced from a FVIII sample by affinity
30 chromatography on monoclonal 56 IgG Sepharose (produced as stated by O. Nordfang et al.: Thrombosis and Haemostasis, Vol. 54, p. 586-590, 1985. 56 IgG Sepharose binds the FVIII-LC/FVIII-HC complex via FVIII-LC. 25 ml of FVIII sample with a content of 405 FVIII-HCAg
35 units/ml were incubated overnight with 1.5 ml of 56 IgG Sepharose (coupled with 4 mg of 56 IgG/ml). The

incubation mixture was poured on a column, and eluate was collected. The gel was washed with 5 ml of buffer B (20 mM imidazole/0.15 M NaCl/10% glycerol/0.1 M lysine/pH 7.4) containing 0.35 M CaCl_2 . Then the gel was washed with 15 ml of buffer B with a total NaCl content of 0.65 M followed by 5 ml of buffer B with 10 mM EDTA and 0.02% NaN_3 (EDTA buffer). The gel was drained and incubated for 1 hour at room temperature with EDTA buffer. After incubation, FVIII-HC was eluted with 5 ml of EDTA buffer. A peak fraction of 2 ml was dialysed to 50 mM imidazole/0.15 M NaCl/10% glycerol/0.02% NaN_3 /pH 7.4. The content of FVIII components in the dialysed sample appears from table 1.

TABLE 1

FVIII fragments in dialysed FVIII-LC sample and FVIII-HC sample

	FVIII:C unit/ml	FVIII-LCAg unit/ml	FVIII-HCAg unit/ml
FVIII-LC (sample A)	<0.01	770	1.8
FVIII-HC (sample B)	<0.01	0.1	2000

25

Samples A and B were analysed by SDS-PAGE, see the attached figure, in which

Lane 1 corresponds to sample A (FVIII-LC)
 4 FVIII-LCAg units,
 Lane 2 contains molecular weight markers, and
 Lane 3 corresponds to sample B (FVIII-HC)
 8 FVIII-HCAg units.

35 The process of the invention will be illustrated below by means of some working examples.

EXAMPLE 1

Samples A and B were each diluted 10 times in buffer C (50 mM imidazole, 0.15 M NaCl, 0.1% BSA, pH 7.4).

- 5 20 μ l of A (diluted 1:10) was mixed with 20 μ l of B 1:10, 3 μ l of 0.15 M MnCl₂ and 40 μ l of buffer C. After 48 hours' incubation at 22°C the incubation mixture contained 1200 m units of VIII:C/ml, measured by Coatest.

10. The following experiments were performed for comparison purposes

Experiment A:

- 15 The experiment was repeated as described in the example with the change that 3 μ l of buffer C were added instead of 3 μ l 0.15 M MnCl₂. After 48 hours' incubation at 22°C, this incubation mixture contained less than 5 m units of VIII:C/ml, measured by Coatest.

20

Experiment B:

Sample A was diluted 40 times in buffer C. 80 μ l of A (diluted 1:40) were mixed with 3 μ l of 0.15 M MnCl₂.

- 25 After 48 hours' incubation, the incubation mixture contained less than 5 units of VIII:C/ml. Similarly, an incubation mixture with sample B contained less than 5 m units of VIII:C/ml.

- 30 Experiment C:

80 μ l of FVIII sample diluted 1000 times in buffer C were mixed with 3 μ l of 0.15 M MnCl₂. After 48 hours' incubation 170 m units of VIII:C/ml were measured. 80 μ l

- 35 of FVIII sample diluted 1000 times in buffer C were mixed with 3 μ l of buffer C. After 48 hours' incubation

140 m units of FVIII:C/ml were measured.

EXAMPLE 2

- 5 Samples A and B were each diluted 10 times in buffer C. 20,ul of A 1:10 were mixed with 20,ul of B (diluted 1/10), 3,ul of 2.2 M CaCl_2 and 40,ul of buffer C. After 12 days' incubation at 22°C, the incubation mixture contained 1300 m units of VIII:C/ml.

10.

The following experiment was performed for comparison purposes

- 15 The test was repeated as described above, but with the change that 3,ul of buffer C were added instead of 3,ul of 2.2 M CaCl_2 . After 12 days' incubation at 22°C, the incubation mixture contained less than 5 m units of VIII:C/ml.

20 EXAMPLE 3

- Samples A and B were each diluted 100 times in buffer C. 100,ul of A and 100,ul of B were mixed. 50,ul of the mixture were tested in Coatest, as described above.
- 25 1.5 m units/ml were measured in the mixture. 50,ul of the mixture were moreover tested in modified Coatest with 1 hour's preincubation with FIXa/FX prior to addition of PL. Hereby, the Coatest activity increased to 3.0 m units/ml.

30

The following experiment was performed for comparison purposes

- 35 FVIII sample was diluted 30,000 times in buffer C. 50,ul of diluted sample were tested in Coatest, as described above. 3.4 m units/ml were measured.

50 μ l of the diluted FVIII sample were moreover tested in modified Coatest with 1 hour's preincubation with FIXa/FX prior to addition of PL. 3.8 m units/ml were measured for the diluted FVIII sample.

5

EXAMPLE 4

When performing an experiment as described in example 1, 1000 m units of VIII:C/ml were measured after 24
10 hours' incubation. When 40 μ l of FIXa/FX were added instead of 40 μ l of buffer C, 1600 m units/ml were measured after 24 hours' incubation.

The following experiment was performed for comparison
15 purposes

The experiment was performed as described in example 1, first comparison experiment. After 24 hours' incubation less than 5 m units/ml were measured in the incubation
20 mixture.

Another experiment for comparison purposes was performed as follows:

25 40 μ l of FVIII sample diluted 500 times in buffer C were mixed with 40 μ l of FIXa/FX and 3 μ l of 0.15 M Mn^{2+} . After 24 hours' incubation, 120 m units/ml were measured. When 40 μ l of FIXa/FX were replaced by 40 μ l of buffer C, 170 m units/ml were measured. When addi-
30 tionally the 3 μ l of 0.15 M Mn^{2+} was replaced by 3 μ l of buffer C, 140 m units/ml were measured.

EXAMPLE 5

35 Samples A and B were each diluted 20 times in buffer C. 20 μ l A 1/20 were mixed with 20 μ l B 1/20, 40 μ l

FIXa/FX and 3 μ l 0.15M CaCl_2 . After 4 hours' incubation at 22°C the incubation mixture contained 199 mU of FVIII:C/ml. If FIXa/FX in the mixture was replaced by 40 μ l buffer C, then 43 mU of FVIII:C/ml were measured after 4 hours' incubation.

EXAMPLE 6

FVIII-LC and FVIII-HC samples containing 800 units of FVIII-LC:Ag/ml and 850 units of FVIII-HC:Ag/ml respectively, were each diluted 3 times. 20 μ l of FVIII-LC 1/3 were mixed with 20 μ l of FVIII-HC 1/3 and 10 μ l of Me^{2+} . In mixture A, Me^{2+} was 25 mM Mn^{2+} . In mixture B, Me^{2+} was 250 mM Ca^{2+} , and in mixture C, Me^{2+} was 25 mM Mn^{2+} and 250 mM Ca^{2+} . After 24 hours' incubation, mixture A contained 10.3 units of FVIII:C/ml, mixture B contained 4.0 units and mixture C contained 12.9 units of FVIII:C/ml. After 144 hours' incubation mixtures A, B and C contained 6.6 units, 6.5 units and 11.9 units of FVIII:C/ml, respectively.

EXAMPLE 7

COS cells were transfected with plasmid pSVF8-80 which expresses 80 kD chain, cf. DK Patent Application 0428/87. Supernatant from the culture containing 870 m units of FVIII-LC:Ag/ml was supplemented with plasma-purified FVIII-HC to a final concentration of 20 FVIII-HC:Ag units/ml and Mn^{2+} to a final concentration of 5 mM. After 24 hours' incubation at 22°C the mixture contained 137 m units of FVIII:C/ml. When plasma-purified FVIII-LC at a concentration of 1000 m units of FVIII-LC:Ag/ml was correspondingly supplemented with FVIII-HC and Mn^{2+} , the incubation mixture contained 33 m units of FVIII:C/ml after 24 hours' incubation. When the culture supernatant was supplemented with only Mn^{2+} and not FVIII-HC, the

mixture contained less than 2.5 m units of FVIII:C/ml after incubation for 24 hours.

EXAMPLE 8

5

25 μ l of FVIII-LC were mixed in buffer C with 25 μ l of FVIII-HC, 7 μ l of $MnCl_2$ and 10 μ l of redox agent to obtain end concentrations of the individual components as stated in table 2. FVIII:C was measured after 5 hours' incubation at 20°C.

TABLE 2

Recombination of FVIII-LC and FVIII-HC in the presence of a redox agent.

	FVIII-LC:Ag units/ml	FVIII-HC:Ag units/ml	mM $MnCl_2$	Redox agent	% FVIII:C in relation to FVIII-LC:Ag after 5 hours
20	11	11	5	none	4.9
	11	11	5	15 μ M DTT	16.8
	11	11	5	150 μ M ME	18.8
	11	11	5	30 μ M Cys	17.4
	11	112	5	15 μ M Cys	68.8
25	11	11	0	none	<0.5

At the stated concentrations of DDT (dithiotreitol), ME (mercapto ethanol) and Cys (Cystein), there is equilibrium between oxidizing and reducing form in aqueous buffer.

EXAMPLE 9

FVIII-HC was produced as described above and with the modification that EDTA elution buffer and dialysis buffer

were mixed with 50 μ m of mercapto ethanol. After dilution recombination with FVIII-HC samples was performed with 20 hours' incubation in buffer C at room temperature with addition of mercapto ethanol to 35 μ M, MnCl_2 to 5 mM and FVIII-LC to 22 FVIII-LC:Ag units/ml.

Table 3 shows recombination with the two FVIII-HC sample types.

10 TABLE 3

FVIII-HC	FVIII-HC:Ag units/ml in recombination mixture	% FVIII:C in relation to FVIII-HC:Ag after 20 hours
15 Sample 1, produced as described above	2.59	31
Sample 2, produced as described above	3.31	27
20 Sample 3, produced with ME as stated in this example	3.00	89

EXAMPLE 10

25 An incubation mixture of the following composition was produced in buffer C from separated FVIII fragments: 60 units/ml of FVIII-LC, 60 units/ml of FVIII-HC, 50 mM CaCl_2 , 2 units/ml of FX.

30 After 20 hours' incubation at room temperature, 6.9 FVIII:C units/ml were measured. In a corresponding incubation mixture without FX, 0.59 FVIII:C unit/ml was measured after 20 hours' incubation at 20°C.

P A T E N T C L A I M S

1. A process for producing a coagulation active complex of an N terminal fragment of Factor VIII with a molecular weight of 92 to 210 kD and a C terminal fragment of Factor VIII with a molecular weight of 80 to 70 kD, characterized by causing the two fragments to react with each other in the presence of a complex promoting agent.
2. A process according to claim 1, characterized in that the N terminal fragment is a heavy chain with a molecular weight of about 92 kD, and that the C terminal fragment is a light chain with a molecular weight of about 80 kD.
3. A process according to claim 1 or 2, characterized in that one fragment or both fragments are derivatized.
4. A process according to claim 3, characterized in that the derivatization comprises replacement of one or more cys-amino acids by another amino acid, preferably serine.
5. A process according to claim 3, characterized in that an FVIII heavy chain and/or FVIII light chain is treated with a substance having reactivity to compounds containing the group -SH and/or -S-S-.
6. A process according to claims 1-5, characterized in that one fragment or both fragments are produced biosynthetically.
7. A process according to any of claims 1-6,

c h a r a c t e r i z e d in that the complex promoting agent is one or more divalent metal ions.

8. A process according to claim 7,
5 c h a r a c t e r i z e d in that the complex promoting agent is Mn^{2+} , Ca^{2+} or Co^{2+} .

9. A process according to any of claims 1-6,
c h a r a c t e r i z e d in that the complex promoting
10 agent is a component of the prothrombin complex.

10. A process according to any of claims 1-6,
c h a r a c t e r i z e d in that the complex promoting agent is FIX, FIXa, FX, FXa or a mixture of two or more
15 of these components.

11. A process according to any of claims 1-6,
c h a r a c t e r i z e d in that the complex promoting agent is von Willebrand Factor.
20

12. A process according to any of claims 1-6,
c h a r a c t e r i z e d in that the complex promoting agent is a divalent metal ion and a component of the prothrombin complex, preferably FX.
25

13. A process according to any of claims 1-6,
c h a r a c t e r i z e d in that the complex promoting agent is a substance having reactivity to components containing the group -SH and/or -S-S-.
30

14. A process according to any of claims 1-6,
c h a r a c t e r i z e d in that the complex promoting agent is a mixture of a divalent metal ion and a substance having reactivity to compounds containing the group
35 -SH and/or -S-S-.

15. A process according to claim 13,
c h a r a c t e r i z e d in that the complex promoting
agent is a protein condensation reagent, such as glutar-
aldehyde, hydrazine, bisepoxiranes, epichlorohydrine,
5 divinylsulfone, benzoquinone, carbonyldiimidazole or
carbodiimide.

10

15

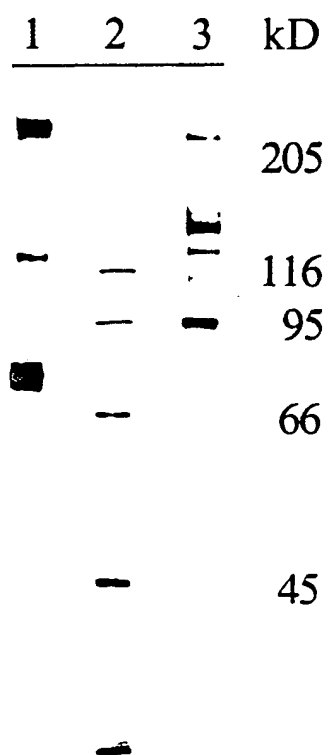
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INTERNATIONAL SEARCH REPORT

International Application No PCT/DK87/00080

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC ⁴		
C: 07 K 15/06		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
IPC	A 61 K 35/14, /16, 37/02; C 07 G 7/00; C 07 K 3/08, 3/10, 13/00, 15/00, /02, /06, /12	
US C1	260: 112; 424: 101; 514: 2, 21; 530: 383	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
SE, NO, DK, FI classes as above		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
A	EP, A1, 0 123 945 (SCRIPPS CLINIC AND RESEARCH FOUNDATION) 7 November 1984	1-15
A	EP, A2, 0 182 372 (SCRIPPS CLINIC AND RESEARCH FOUNDATION) 28 May 1986	1-15
P, A	EP, A1, 0 197 901 (KABIVITRUM AB) 15 October 1986	1-15
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Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
1987-09-17	1987-09-23	
International Searching Authority	Signature of Authorized Officer	
Swedish Patent Office	Elisabeth Carlborg	

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